Amendments to the Specification

Replace the paragraph beginning at page 4, line 15, with the following paragraph:

The invention also features isolated DNA encoding a DAF-2 polypeptide. This isolated DNA can have a nucleotide sequence that includes, for example, the nucleotide sequence of the *daf*-2 gene shown in ~~Fig. 2B~~ Figs. 2B-1, 2B-2, and 2B-3. This isolated DNA can also, in preferred embodiments, complement a *daf-2* mutation in *C. elegans*, an InR mutation in a mouse, or an IGF-1 receptor mutation in a mouse.

Replace the paragraph beginning at page 5, line 10, with the following paragraph:

The invention also features a method of detecting a gene, or a portion of a gene, that is found in a human cell and has sequence identity to the *daf-2* sequence of ~~Fig. 2B~~ Figs. 2B-1, 2B-2, and 2B-3. In this method, isolated DNA encoding a DAF-2 polypeptide, a portion of such DNA greater than about 12 residues in length, or a degenerate oligonucleotide corresponding to SEQ ID NOS: 33, 34, 79, 80, 81, 82, 83, or 84, is contacted with a preparation of DNA from the human cell under hybridization conditions that provide detection of DNA sequences having about 70% or greater nucleic acid sequence identity to the *daf-2* sequence of ~~Fig. 2B~~ Figs. 2B-1, 2B-2, and 2B-3. This method can also include a step of testing the gene, or portion thereof, for the ability to functionally complement a *C. elegans daf-2* mutant.

Replace the paragraph beginning at page 6, line 9, with the following paragraph:

The invention also features isolated DNA encoding a DAF-3 polypeptide. This isolated DNA can have a sequence that includes, for example, the nucleotide sequence of a *daf-3* gene shown in Figs. ~~11A, 11B, or 11C~~ 11A-1, 11A-2, 11B-1, 11B-2, 11C-1, or 11C-2. This isolated DNA can also, in preferred embodiments, complement a *daf-3* mutation in *C. elegans* or complement a *daf-3* knockout mouse.

Replace the paragraph beginning at page 7, line 4, with the following paragraph:

The invention also features a method of detecting a gene, or a portion of a gene, that is found in a human cell and has sequence identity to any of the *daf-3* sequences of Figs. ~~11A, 11B, or 11C~~ 11A-1, 11A-2, 11B-1, 11B-2, 11C-1, or 11C-2. In this method, isolated DNA encoding a DAF-3 polypeptide, a portion of such DNA that is greater than about 12 residues in length, or a degenerate oligonucleotide corresponding to SEQ ID NOS: 35, 36, or 85, is contacted with a preparation of DNA from the human cell under hybridization conditions that provide detection of DNA sequences having about 70% or greater nucleic acid sequence identity to any of the *daf-3* sequences of Figs. ~~11A, 11B, or 11C~~ 11A-1, 11A-2, 11B-1, 11B-2, 11C-1, or 11C-2. This method can also include a step of testing the gene, or portion thereof, for the ability to functionally complement a *C. elegans daf-3* mutant.

Replace the paragraph beginning at page 7, line 15, with the following paragraph:

Another method included in the invention is a method of isolating a gene, or a portion thereof, that is found in a human cell and has at least 90% nucleic acid sequence identity to a sequence encoding SEQ ID NOS: 35, 36, or 85. This method includes (a) amplifying by PCR the human gene, or portion thereof, using oligonucleotide primers that (i) are each greater than about 12 residues in length, and (ii) each have regions of complementarity to opposite DNA strands in a region of any of the nucleotide sequences of Figs. ~~11A, 11B, or 11C~~ 11A-1, 11A-2, 11B-1, 11B-2, 11C-1, or 11C-2, and (b) isolating the human gene, or portion thereof. This method can also include a step of testing the gene, or portion thereof, for the ability to functionally complement a *C. elegans daf-3* mutant.

Replace the paragraph beginning at page 32, line 26, with the following paragraph:

~~**Fig. 2B** shows~~ **Figs. 2B-1, 2B-2, and 2B-3** show the cDNA encoding the *C. elegans* DAF-2.

Replace the paragraph beginning at page 33, line 1, with the following paragraph:

~~**Fig. 2C shows**~~ **Figs. 2C-1 and 2C-2** show the amino acid comparison of *C. elegans* DAF-2 to the human insulin receptor and human IGF-I receptor (shown in parenthesis), and to the *Drosophila* insulin receptor homolog, with *daf-2* and human insulin receptor mutations highlighted. Six *daf-2* mutations map in the ligand-binding domain: *sa187* (C347S, TGT to AGT), *e1368* (S451L, TCA to TTA), *e1365* (A458T, GCT to ACT), *sa229* (D526N, GAT to AAT), and two mutations in *mg43* (C279Y, TGT to TAT and P348L, CCC to CTC). Three *daf-2* mutations substitute conserved amino acid residues in the insulin receptor kinase domain: *sa219* (D1252N, GAT to AAT), *e1391* (P1312L, CCC to CTC), and *e1370* (P1343S, CCA to TCA). Darkened residues indicate amino acid identity. Hatched residues indicate amino acid similarity. The percentages under the domains represents the percentage of identity observed between DAF-2 and each receptor. The corresponding BLAST probabilities of DAF-2 random match to each protein is: 6.4×10^{-157} (human insulin receptor), 2.7×10^{-156} (human IGF-I receptor), 2.1×10^{-153} (molluscan InR homolog), 8.3×10^{-153} (mosquito InR homolgoue), 1.6×10^{-138} (human insulin receptor-related receptor), $1.7x\ 10^{-122}$ (*Drosophila* InR homolog), 2.0×10^{-108} (Hydra InR homolog). DAF-2 is more distant from the next most closely related kinase families: 8.9×10^{-58} (v-ros) and 3.0×10^{-51} (trkC neurotrophin receptor).

Replace the paragraph beginning at page 38, line 16, with the following paragraph:

Figs. ~~11A-11C~~ 11A-1, 11A-2, 11B-1, 11B-2, 11C-1, and 11C-2 show the cDNA sequences of the differentially spliced *C. elegans daf-3* transcripts (SEQ ID NOS: 39, 52, and 53).

Replace the paragraph beginning at page 38, line 24, with the following paragraph:

~~**Fig. 15 shows**~~ Figs. 15-1 and 15-2 show the cDNA sequence of the

C. elegans age-1 gene (SEQ ID NO: 47).

Please replace the paragraph beginning at page 39, line 9, with the following paragraph:

~~**Fig. 21A is an illustration**~~ Figs. 21A-1, 21A-2, and 21A-3 are illustrations showing that human FKHR and AFX are the closest relatives to DAF-16. Note that the differentially spliced DAF-16 forkhead domain is less homologous.

Replace the paragraph beginning at page 43, line 3, with the following paragraph:

The amino acid sequences and nucleotide sequences encoding DAF-2 are shown in Figs. 2A and ~~2B~~ 2B-1, 2B-2, and 2B-3, respectively. Using BLASTX to compare 570 translated Y53G8 M13 subclone sequences against the Genbank protein database, we found that four sequences are homologous to the mammalian insulin receptor family. An insulin receptor was a good *daf-2* candidate gene because insulin regulates vertebrate growth and metabolism (White and Kahn, *J. Biol. Chem.* 269: 1-4, 1994), and because a phosphatidylinositol (PI) 3-kinase has been shown to act in both the insulin receptor and *daf-2* pathways (White and Kahn, *J. Biol. Chem.* 269: 1-4, 1994; Morris et al., *Nature* 382: 536-539, 1996). The detection of multiple *daf-2* mutations in the gene (see below), and the coincidence of the genetic location of this insulin receptor homolog with *daf-2* (~~Fig. 2C~~ Figs. 2C-1 and 2C-2) establish that this insulin receptor homolog corresponds to *daf-2*.

Replace the paragraph beginning at page 44, line 9, with the following paragraph:

In the approximately 500 amino acid ligand-binding domain of the insulin receptor, DAF-2 is 36% identical to insulin receptor and 35% identical to the IGF-I receptor. Twenty-one of twenty-three phylogenetically conserved cysteine residues in this domain are conserved

in DAF-2 (~~Fig. 2C~~ Figs. 2C-1 and 2C-2). The DAF-2 cys-rich region is 34% identical to human insulin receptor and 28% identical to the IGF-I receptor. Six *daf-2* mutations map in this domain (~~Fig. 2C~~ Figs. 2C-1 and 2C-2, Table I). The *mg43* and *sa187* mutations substitute conserved residues in the cys-rich region (~~Fig. 2C~~ Figs. 2C-1 and 2C-2). *daf-2(mg43)* carries two mutations which substitute conserved residues, which may explain the strength of this allele (non-conditional, Table I). Other substitutions at non-conserved residues cause less severe phenotypes (Table I). Insulin resistant and diabetic patients with mutations in the ligand binding domain of the human insulin receptor gene have been identified (Taylor, *Diabetes* 41: 1473-1490, 1992) (see below). These mutations impair receptor transport to cell surface, or insulin binding affinity, or both. The DAF-2 mutations in this domain might similarly decrease receptor signaling to cause dauer arrest.

Replace the paragraph beginning at page 45, line 2, with the following paragraph:

The 275 amino acid DAF-2 tyrosine kinase domain is 70% similar and 50% identical to the human insulin receptor kinase domain. Upon insulin binding, the intracellular tyrosine kinase domain of the insulin receptor phosphorylates particular tyrosine residues flanked by signature amino acid residues (upstream acidic and downstream hydrophobic amino acids (Songyang and Cantley, *Trends Biochem. Sci.* 20: 470-475, 1995)) in the intracellular domain as well as on IRS-1 (White and Kahn, *J. Biol. Chem.* 269: 1-4, 1994). Multiple DAF-2 tyrosine residues in these sequence contexts are likely autophosphorylation targets, including three tyrosines in a region similar to the insulin receptor activation loop and one in the juxtamembrane region as described above (~~Fig. 2C~~ Figs. 2C-1 and 2C-2). Based on the crystal structure of the insulin receptor kinase domain bound to its activation loop, eight kinase domain residues mediate target site specificity (Hubbard et al., *Nature* 372: 746-754, 1994). In DAF-2 (but not in more distantly related receptor kinases), these residues are invariant (5/8) or replaced with similar amino acids (3/8: K to R, E to D) (~~Fig. 2C~~ Figs. 2C-1 and 2C-2), suggesting that DAF-2 phosphorylates the same target tyrosine motifs as the insulin receptor kinase.

Replace the paragraph beginning at page 45, line 18, with the following paragraph:

Three *daf-2* missense mutations substitute conserved amino acid residues in the kinase domain (~~Fig. 2C~~ Figs. 2C-1 and 2C-2, Table I). All three mutations cause moderate to strong dauer constitutive phenotype, but none are as strong as the non-conditional alleles, for example, *mg43* (Table I). Human insulin receptor mutations in the kinase domain exhibit decreased kinase activity and cause severe insulin resistance and associated defects (Fig. 2C; Taylor, *Diabetes* 41: 1473-1490, 1992). Remarkably, a human diabetic insulin resistant patient bears the same amino acid substitution (P1178L) as *daf-2(e1391)* (Kim et al., *Diabetologia* 35: 261-266, 1992). This patient was reported to be heterozygous for this substitution. *daf-2(e1391)* is not dominant whereas it is a highly penetrance recessive mutation (Table I).